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STUDIES ON CHROMOLYTIC EFFECTS OF THE  
 CEREBROSPINAL FLUID.

by

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1 Introduction

(1) Review of the literature.

The ultraviolet ray absorption spectrum of the cerebrospinal fluid in cases of cerebral concussion shows marked selective absorption bands with a peak at 265 millimicrons (M. Spiegel-Adolf, E. A. Spiegel, H. T. Wycis; 1946). This is an indication of the appearance of nucleic acids or their derivatives in the cerebrospinal fluid (C. S. F.). When the fluid is left standing aseptically, the selective absorption bands gradually weaken and finally disappear, due to the action of chromatolytic enzymes. If nucleic acids are added to C. S. F. of a patient of cerebral concussion and to that of a normal man and both fluids are kept at 37°C, the former shows a decrease of absorption bands of nucleic acids, whereas the latter undergoes almost no change. The decrease is supposed to be the result of the appearance of an enzymatic substance decomposing nucleic acids and their derivatives in C. S. F. after cerebral concussion. The enzyme comes probably from the central nervous system and may be responsible for the decrease or disappearance of Nissl bodies (nucleic acid substance) in nerve cells after cerebral concussion.

Particular practice in technique and expensive apparatus required for ultraviolet spectrophotometry makes its use very difficult in ordinary laboratories. Thus Spiegel and others turned their attention on nucleic acids constituting Nissl bodies of nerve cells and tried to demonstrate histologically the change of staining characteristics of Nissl bodies due to the action of a chromatolytic enzyme in the fluid. In their method, paraffin sections of the spinal cord of a normal cat, mounted on slide glasses, were surrounded by thick square paraffin frames. The inside of the frames was filled with the C. S. F. to be tested (made acidic by acetic acid), covered with a cover glass and kept for four hours at 37°C. Then the frames were

taken off and Nissl's staining was done with 0.1% thionine blue solution. By enzymatic effects of C. S. F., which had been taken three days after cerebral concussion. Nissl bodies faded and the cytoplasm of anterior horn cells was stained homogeneously.

On the contrary, in the sections treated with Ringer's solution or in the control sections untreated, Nissl bodies were distinctly and densely stained. One year and a half after cerebral concussion the C. S. F. showed no tigrolysis.

Quite the same were the results obtained by ultraviolet spectrophotometry.

The tigrolytic action of the fluid is said to be recognized even in patients of cerebral concussion with only slight clinical signs.

The above is the summary of the report of E. A. Spiegel and others (1946). It is well-known that tigrolysis occurs in nerve cells when some injuries are inflicted on the central nervous system. If Spiegel is correct, it is possible that the tigrolysis is related with the appearance of tigrolytic enzymes in C. S. F.. Since Nissl's staining technique is routinely used in a clinical laboratory, Spiegel's method will aid us much in the diagnosis of head injury.

Caspersson (1941) recognized that Nissl body of nerve cells is principally composed of ribonucleic acid. However no method practicable in clinical laboratories is available at present for the quantitative determination of this nucleic acid. For this purpose Nissl's staining technique is inaccurate (Hyden 1947, Fulton 1951) and there are not infrequently some disagreements between the quantitative value estimated morphologically and that determined by an elaborate biochemical method (Shibatani 1953, Harrington 1951). This may be due to the fact that nucleic acids do not maintain a definite form in living cells (Mott, Marinesco 1912) and remain as viscous solution (Berg 1912) and that tigroid figures are formed by sedimentation after alcohol fixation or acidification of the medium (Held 1895, Scheinin 1932, Bensley 1933, Hopkins 1924).

But it is definitely established that Nissl bodies are not stained by basic dyes when treated with ribonuclease (Brachet 1940, Davidson, Waymouth 1944). Moreover Spiegel's method is practical and is said to give the results, which are in accord with studies on absorption bands of ultraviolet ray. Thus I have attempted to reexamine the method.

## (2) Reexamination of Spiegel's method.

Following Spiegel's description I have made the examination. Tigrolytic effect is observed in C. S. F. of the patients of cerebral injuries, but not in Ringer's solution. In the former (Fig. 3), nuclear membrane and nucleolus of nerve cells of the spinal cord are remaining, but Nissl body has disappeared completely. The cytoplasm is homogeneously of faintly red colour. The nuclei of glia cells in the neighbourhood are normally stained. The findings are in distinct contrast with those in control examination (Fig. 1).

However it should be noted that tigrolysis can sometimes occur by the action of C. S. F. of the patients who have apparently no abnormalities in the central

nervous system. Spiegel refers to this point and states that the regulation of pH of C. S. F. between 7.0-7.4 prevents this phenomenon. But in this pH range of the fluid tigrolysis is hindered from occurring also in cases of cerebral injuries. An adequate pH value at which tigrolysis takes place specifically in cases of cerebral injury, can not be established, because of individual variations. If even a slight tigrolysis is regarded as being positive, there may be some differences between the C. S. F. of a normal person and that of a cerebral injured. But a considerable skill is required to be able to recognize a slight tigrolysis with confidence; this can differ from one examiner to another. Therefore I have decided to regard complete disappearance of Nissl body only as positive tigrolysis, thus some discrepancies being inevitable between the results of Spiegel and mine.

The tigrolysis in celloidine sections of the spinal cord of a normal cat has been compared with that in paraffin sections of the same spinal cord. No difference was found between the two in 20 fluids, with only one exception. The same was true with the fluids diluted by multiplication which are to be described later. The staining of Nissl body seems to be more difficult in paraffin sections than in celloidine sections. Also the procedure of treating the sections with C. S. F. is much easier in the latter than in the former (to be described in detail later). Therefore I used to make celloidine sections only.

The optimal time required for the treatment with C. S. F. was investigated in twelve cases. Tigrolysis occurred in an hour and a half in one case, two hours in two cases, two hours and a half in two cases, three hours in two cases and no tigrolysis occurred in four to six hours in five cases. Therefore tigrolysis should occur, if it is positive, within hours and tigrolysis is negative, if it does not occur even after more than four hours; i. e. the time necessary for the positive reaction is four hours at longest.

The temperature during the reaction is 37°C. ; 60°C shortens the time for the reaction to one hour, but this temperature tends to fade the colour of thionine at the time of alcohol differentiation and therefore has not been used in the experiment. Spiegel reports that the satisfactory staining can be obtained also at room temperature (23°C~25°C) for 17-19 hours.

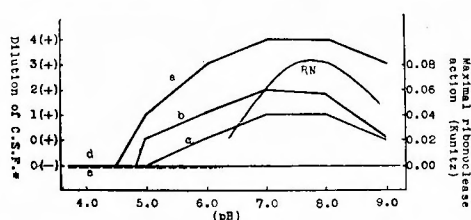
pH value of the test fluid was corrected by acetate buffer or by carbonate buffer. Experiments at various pH values show that in deparaffinized paraffin sections, corbowax sections and frozen sections, tigrolysis occurs at any pH value of the fluid above 6.0 regardless whether the fluid is taken from a normal person or from a cerebral injured. Besides it occurs in the same way also in the control medium; e.g. distilled water or Ringer's solution of the same pH. But in celloidine sections all the control fluid show negative tigrolysis at pH values ranging from 1.6 to 10.8. But at pH values above 6.0, cells in general are too densely stained and even the celloidine substance around the tissue is stained with the dye.

The above mentioned phenomenon may be understood by the assumption that Nissl body (nucleic acid substance) loses its characteristic form, becoming solution-

like at pH values above 6.0, and retains its form by sedimentation, only when it is acidic. In case of celloidine sections embedding medium is remaining at the time of staining and prevents the liquefaction of Nissl body, while in deparaffinized sections embedding medium is lost at staining. Thus celloidine sections are superior to other method of embedding in that chemical changes of Nissl body can be studied in fairly wide pH ranges of the fluid without being confused by physical effects.

When examined in celloidine sections, the C. S. F. which is tigrolysis-positive at higher pH values, is tigrolysis-negative at lower pH values, particularly below 4.5. This may be due to the inactivity of nuclease at pH values below 4.5.

In this connection, I have made, for the purpose of determining the intensity of enzymatic action, multiple dilution of C. S. F. with physiological saline solution.



**Table 1.** Tigrolytic effect of C. S. F. in every pH value.

a, b, c, : Maximal dilution (multiple dilution 1:2) of tigrolysis-positive C. S. F. at each pH. Examinations in three tigrolysis-positive cases (a, b, c.)

d : Uninterrupted line : Maximal dilution in control (tigrolysis-negative) fluid in celloidine sections.

e : Interrupted line : Maximal dilution in control (tigrolysis-negative) fluid in carbowax or paraffin sections.

RN : Action curve of ribonuclease in various pH values (Kunitz, 1940)

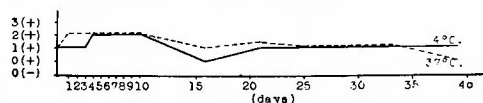
\* 0, 1, 2, 3, 4 mean 2<sup>0</sup>, 2<sup>1</sup>, 2<sup>2</sup>, 2<sup>3</sup>, 2<sup>4</sup> respectively.

sections of the cat's spinal cord and 0.5 cc. of the fluid to be tested. If two or four sections are put into the fluid at the same time, the sections turn out to be tigrolysis-positive, whereas tigrolysis becomes negative when sixteen sections are put into the fluid at the same time; a fact which shows that this enzyme activity does not follow all-or-nothing formula, but that it varies quantitatively to a certain extent.

Next, the C. S. F. preserved immediately after puncture in an icebox (4°C) and that in an incubator (37°C), have been tested for the change of enzymatic activity

C. S. F. of a given pH value was diluted successively to 1:2 and all samples were tested for tigrolysis in celloidine sections. Maximal dilution of C. S. F. in which tigrolysis took place, was found out at various pH values and the curve in Table 1 was obtained. It shows that enzyme action has its maximum effect at pH 7.0-8.0, decreasing gradually at pH values below or above this range. This is roughly in agreement with the fact that optimum pH of ribonuclease action is 7.7. Thus comparison of the intensity of enzymatic activities of various C. S. F. should be made at pH 7.7. However at pH values above 6.0 precise recognition of tigrolysis is difficult because of too heavy staining of tissues. Therefore I have adopted pH 5.0 and 6.0 as the standard for the comparison of the enzymatic action of C. S. F..

Now I take 20 micra thick celloidine



**Table 2.** Change in nuclease action of tigrolysis-positive C. S. F. when it is kept in either ice box or in incubator.

Uninterrupted line : C. S. F. kept at 4°C (sterile)

Interrupted line : C. S. F. kept at 37°C (sterile)

with the lapse of time (Table 2).

In the former fluid the activity begins to rise on the third day and turns down on the tenth with the minimum on the fourteenth day, returning to the initial value on the twenty first. In nine cases there were no changes in the value until the third day, and in four cases, no changes until the sixth day. In the C. S. F. kept at 37°C the activity rises after twenty four hours, the further course being the same as in the C. S. F. kept in an ice-box. This shows that C. S. F. should be kept in an ice-box immediately after puncture and examined within three days.

After the foregoing preliminary experiments had been done, I examined the tigrolytic activity of C. S. F. in cases of various nervous diseases.

## 2. Detailed description of the methods of investigation.

(1) Taking of C. S. F. : C. S. F. was taken by lumbar, cysternal and ventricular punctures, and by subarachnoideal puncture during craniotomy and through a draining vinyl tube inserted into a dead cavity that was formed after extirpation of a tumor, etc.. C. S. F. was immediately kept aseptically in an ice-box and examined within following three days. The examinations were done in more than five hundred cases in total.

### (2) Spinal cord sections

Normal cats weighting two to three kilograms were killed by bleeding from the carotid artery on one side. The spinal cords were taken out in their entire length, washed with seventy percent alcohol, immediately afterwards fixed with ninety percent alcohol, transferred to 99.5% alcohol on the next day to be fixed for a week and then placed in 100% alcohol for a week. Celloidine embedding was done after the completion of fixation. 20 micra thick transverse sections of the spinal cords were made and preserved in 70% alcohol.

### (3) Buffer solutions

The buffer solutions used in the experiment were as follows:

1)	M/5 acetate buffer	pH 4.05
	M/5 acetic acid	120cc.
	M/5 sodium acetate	24cc.
2)	1M acetate buffer	pH 4.05
	1M acetic acid	120cc.
	1M sodium acetate	24cc.
3)	1N hydrochloric acid buffer	pH 1.42
	1N sodium acetate	25cc.
	1N hydrochloric acid	30cc.
	Distilled water	70cc.
4)	0.1M carbonate buffer	pH 10.16
	0.1M sodium carbonate	50cc.
	0.1M sodium bicarbonate	30cc.

Among these, M/5 acetate buffer was principally used.

#### (4) Technique of examination

10 measurement bottles (2cc.) are arranged in a row. First 0.9cc. of C. S. F. and next 0.15cc. of M/5 acetate buffer are put into the first bottle at the left end of the row with sterile pipettes and mixed. The remaining 9 bottles are each filled with 0.5cc. of physiological saline. 0.5cc. of the fluid taken from the first bottle at the left end is put into the second bottle, from which 0.5cc. is taken and put into the third. The same is repeated up to the ninth on the right. The tenth bottle at the right end is filled with physiological saline only and serves as a control.

Sections of the spinal cord are taken out of alcohol, quickly washed in water and two sections are put in each bottle. The bottles are covered and kept at 37°C for four hours. Then the sections are taken out, washed in distilled water. Nissl's staining with 0.1% thionine blue, alcohol differentiation, clearing and sealing.

#### (5) Remarks

1) pH 6.0 is attained by adding 0.15cc. buffer solution, but the amount of buffer solution to be added should be regulated, because the pH of C. S. F. varies between 7.6 and 8.2. pH 5.0 was attained by adding 0.2cc. buffer solution. pH test paper of Toyo Roshi Company was conveniently used for pH determination.

2) After correcting pH values, no great changes of pH values were caused by multiple dilution.

3) Alcohol differentiation in this experiment must be done in a much shorter time than in ordinary Nissl's staining, as the colour of thionine tends to fade readily.

4) When Nissl bodies in all anterior horn cells have disappeared, the reaction is regarded as tigrolysis-positive. Physiological saline solution is always taken for control.

### 3. Results of Experiments.

Before investigating C. S. F. of the patients of various nervous diseases, apparently normal C. S. F. in case of hernia inguinalis was tested. At pH 6.0 tigrolysis was found in 2<sup>1</sup>, or 1:2, dilution, and no tigrolysis in 2<sup>2</sup>, or 1:4, dilution. The result is expressed by 6-1 (+), and this mode of expression will be used in the following descriptions. At pH 5.0, tigrolysis sometimes occurs and sometimes does not in 2<sup>0</sup>, or in the original fluid, viz. 5-0 (+) or 5-0 (-).

#### (1) Non-nervous diseases.

43 cases of various diseases other than of the central nervous system were at pH 5.0, tigrolysis-negative; i.e. 5-0 (-) and 34 cases were 5-0 (+). No other values were observed. Efforts were made to find some difference between the tigrolysis-negative and positive groups, regarding the nature of diseases, symptomatology, age, sex, and findings of C. S. F., blood and urine, but no such difference could be found.

At pH 6.0, patients of inguinal hernia, hydrocele testis, appendicitis and diabetes insipidus showed 6-1 (+), but patients of abdominal neurosis, fistula ani, Douglas abscess, ascaridiasis, cholelithiasis and cancers of various organs showed a little



higher value, i.e. 6-2 (+). These 6-2 (+) cases were accompanied by more or less disturbance of hepatic functions.

## (2) Diseases of the central nervous system.

pH 6.0	8	16	<u>26</u>	8
pH 5.0	<u>25</u>	<u>6</u>	1	
	0(-)	0(+)	1(+)	2(+) Dilution*

**Table 3.** Number of cases of brain tumor arranged in the order of tigrolytic intensity of C. S. F.

The columns of underlined numerals correspond to normal value.

\* 0, 1, 2 mean 2<sup>0</sup>, 2<sup>1</sup>, 2<sup>2</sup> respectively

Tigrolytic value*				
6-2 (+)	7	2	10	
6-1 (+)	<u>3</u>	<u>1</u>		<u>1</u>
	4	5	7-34	43 (days in sick-bed)

**Table 4.** Number of cases of encephalitis japonica showing normal or increased tigrolytic effects of C. S. F. on various days in sick-bed.

Underlined numerals indicate the number of cases with normal tigrolytic value.

\*6-2 and 6-1 mean pH 6, dilution 2<sup>2</sup> and pH 6, dilution 2<sup>1</sup> respectively.

orrhage, it was slightly higher, i.e. 6-2 (+) on the second day after bleeding. In cerebral embolism tested two months after the insult, it was 5-0(-).

The value was normal in a great majority of idiopathic epileptics (Table 5), and also in postencephalitic epileptics. But in posttraumatic epilepsy five cases out of eleven gave slightly higher values. In posttraumatic neurosis, seven cases out of eleven showed the value above the normal limit, with the maximum of 6-4 (+). Thus, posttraumatic disorders tended to show higher values. The period elapsing from accident to the time of investigation does not affect the result as is

In brain tumors, at pH 5.0, the results of the test were almost normal as seen in Table 3, most cases showing 5-0. (-). At pH 6.0, about one half of the cases were of normal value and another half were of lower value than normal. Localisation and nature of the tumor were unrelated to the enzymatic value of the fluid.

In 3 cases of meningitis the result was 5-2 (+), 6-2 (+) and 6-4 (+) respectively. In two cases of arachnoiditis one was 6-0 (+), and another was 6-2 (+).

In encephalitis japonica (Table 4), nineteen cases out of twenty four showed 6-2 (+) and five showed 6-1 (+) in the early as well as in the later stage of the disease. All the cases with positive complement fixation reaction showed 6-2 (+).

The value was 6-1 (+) in diseases resembling encephalitis japonica in symptomatology, such as meningism, typhoid fever, and ekiri.

Cases of cerebral abscess showed 5-2 (+). In one case of intracerebral hem-

Posttraumatic disorders	pH 6.0	<u>4</u>	5	1	1
Posttraumatic epilepsy	pH 6.0	<u>6</u>	4	1	
Idiopathic epilepsy	pH 6.0	<u>7</u>	2		
	pH 5.0	<u>9</u>	<u>2</u>	2	
0(-) 0(+) 1(+) 2(+) 3(+) 4(+)					
Nuclolytic value					

**Table 5.** Number of cases of posttraumatic and idiopathic epilepsies and other posttraumatic disorders.

The columns of underlined numerals correspond to normal value.



shown in Table 6.

Posttraumatic disorders	2 <sup>2</sup>	2 <sup>3</sup>	2 <sup>1</sup>	2 <sup>2</sup>	2 <sup>4</sup>	2 <sup>2</sup>	2 <sup>2</sup>	2 <sup>1</sup>										2 <sup>1</sup>
			2 <sup>1</sup>	2 <sup>2</sup>														
Posttraumatic epilepsy			2 <sup>1</sup>					2 <sup>1</sup>	2 <sup>1</sup>	2 <sup>2</sup>	2 <sup>2</sup>	2 <sup>1</sup>	2 <sup>3</sup>	2 <sup>2</sup>		2 <sup>0</sup>	2 <sup>2</sup>	2 <sup>1</sup>
	3	1	3	6	7	13	19	2	3	6	8	9	11	12	14	15	18	19
	w.	m.	m.	m.	m.	m.	m.	y.	y.	y.	y.	y.	y.	y.	y.	y.	y.	y.

**Table 6.** Tigrolytic values of C. S. F. of posttraumatic disorders and posttraumatic epilepsy at various terms after accident.  
In all the cases pH is 6.0, at which normal value is 2<sup>1</sup>. Each numeral represents one case.  
w = week, m = month, y = year.

In neurosis the value was generally higher, viz. 6-2 (+), 6-4(+) and 5-2(+). High values were obtained in many of the psychotic patients. For example, schizophrenia, manic depressive psychosis, taboparalysis, pseudosclerosis and cerebral arteriosclerosis gave 6-3 (+) and 6-4 (+).

Cases of headaches of unknown etiology showed various values ranging from 6-0 (+) to 6-3 (+).

(3) Cranial injuries

I-type of injury (simple type, without cerebral symptoms); Five cases were examined (Table 7), one case

Cranial injury I-type	2 <sup>3</sup>	2 <sup>2</sup>			2 <sup>2</sup>		2 <sup>1</sup>					2 <sup>1</sup>						
II-type	2 <sup>3</sup>	2 <sup>2</sup>	2 <sup>3</sup>				2 <sup>1</sup>			2 <sup>2</sup>		2 <sup>2</sup>			2 <sup>1</sup>			
	2 <sup>0</sup>	2 <sup>2</sup>					2 <sup>1</sup>					2 <sup>2</sup>						
III-type				2 <sup>3</sup>	2 <sup>3</sup>			2 <sup>3</sup>	(2 <sup>3</sup> )	(2 <sup>3</sup> )		2 <sup>3</sup>	(2 <sup>2</sup> )					
IV-type																2 <sup>2</sup>		
																2 <sup>2</sup>		
several	1	2	3	4	5	6	7	8	9	10	11	12	17	24	60	1		
h.	d.	d.	d.	d.	d.	d.	d.	d.	d.	d.	d.	d.	d.	d.	d.	y.		

**Table 7.** Tigrolytic values of C. S. F. in cases of cranial injury, arranged according to the type of injury and the term after injury.  
Numerals in parentheses indicate the values obtained at repeated examinations. Otherwise each numeral represents one case. pH value is 6.0, at which normal value is 2<sup>1</sup>.  
h = hour, d = day, y = year.

investigated two hours after accident gave 6-3 (+). The tigrolytic values of the cases examined 1, 5, 7 and 12 days after accident were 6-2 (+), 6-2 (+), 6-1 (+) and 6-1 (+) respectively, the latter two being normal.

II-type (cerebral concussion type); Twelve cases.

In one case investigated six hours after accident the value was 6-3 (+), in two cases after two days 6-3(+), 6-2 (+), in three cases after one day 6-3 (+), 6-2 (+),

6-0(+), in two cases after twelve days 6-2 (+) and in one case after sixty days 6-1 (+). The results suggest that tigrolytic effects of C. S. F. are increased after accident and normal values are regained after a considerable period of time.

III-type (cerebral contusion type); Four cases.

The first case showed 6-3 (+) after four days, 6-3 (+) after five, 5-3 (+) after seven and 5-3 (+) after seventeen days. The second case gave 6-3 (+) after nine days, the third case 5-3 (+) after eleven, and the fourth case 5-2 (+) after twenty-four days. In this group of patients higher values tended to persist for a long

period.

IV-type (type of delayed appearance of cerebral symptoms); Four cases.

Unexpectedly low values were obtained in two case, viz. 6-0 (+) after five days and 6-2 (+) after six respectively. Remaining two cases with depressed fracture gave 6-2 (+) after one year.

Two cases of epidural hematoma gave 6-3 (+) after two days and 6-3 (+) after thirty-eight days. Of the four cases of chronic subdural hematoma one showed 6-0 (+) after twenty days, one 5-0 (+) after forty-six days, one 5-0 (-) after one hundred and five days and one 6-3 (+) after eight months.

#### (4) After craniotomy

Examination of C. S. F. taken following craniotomy revealed high values, as are shown in Table 8, viz. 6-2 (+), 6-3 (+), 6-4 (+) and 6-6 (+) at the maximum. At pH 5.0, 5-1 (+) was most frequently met with. Worthy of note is

pH 6.0		<u>1</u>	6	9	5	1	1
pH 5.0	<u>1</u>	<u>1</u>	3	1		1	1

0(-) 0(+) 1(+) 2(+) 3(+) 4(+) 5(+) 6(+)

**Table 8.** Number of cases of craniotomy in which the tigrolytic value of C. S. F. was examined after operation.

The columns of underlined numerals correspond to normal value.

After craniotomy				29	6-6(+)*
After iodized oil ventriculography	1		3	17	6-3(+)
After pneumoencephalography	8		4	6	6-2(+)
After mere lumbar-puncture	3		1		(-)
	tigrolytic effect decreased	tigrolytic effect unchanged	tigrolytic effect increased	Maximal rise of tigrolytic effect	

**Table 9** Number of cases of various intracranial interventions arranged according to the kinds of intervention and the changes in tigrolytic effect of C. S. F. after intervention. Maximal rise of tigrolytic effect is given at the right end column of the table.

\* 6-6(+) means positive tigrolysis at pH 6 and dilution 2<sup>6</sup>.

#### time after injury and after craniotomy.

Of the nine cases of brain injury which were followed up interruptedly (Table 10), seven showed a gradual decrease, whereas one showed a decrease on the two-

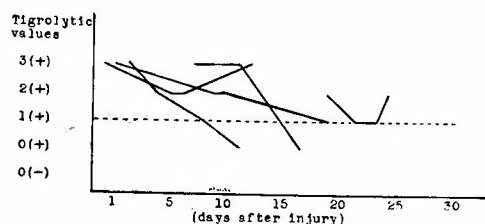
the fact that bloody or xanthochromic C. S. F. tend to show higher values than watery clear C. S. F., but there are some exceptions.

The rate of increase of values after operation is correlated with the magnitude of operation. As seen in Table 9, after craniotomy the values rise in all cases and attain 6-6 (+) at the maximum.

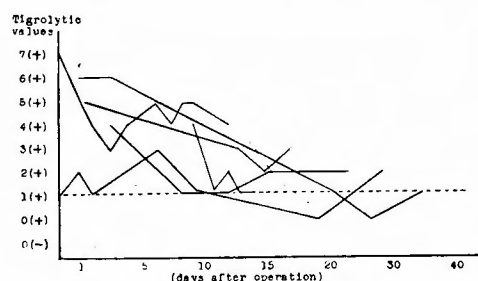
But after iodized oil-ventriculography, though the values rise in most cases (seventeen), the maximum being 6-3 (+), they are the same as before in three cases and drop in one case. After pneumoencephalography by lumbar route, six cases showed a rise, four cases no change, eight cases a drop. The maximum rise was 6-2 (+). Most cases after mere lumbar puncture showed a slight fall. Three cases after electroshock all showed a fall of the values.

#### (5) Changes of the enzymatic values in the course of

nty first day after injury and a rise on the twenty fourth. Also the remaining one



**Table 10.** Changes in tigrolytic effect in the course of time following cranial injury. Dotted line represents normal value.



**Table 11.** Change in tigrolytic effect in the course of time following craniotomy. Dotted line represents normal value.

case gave a higher value on the twelfth day than on the sixth.

In case of brain operation, as is seen in Table 11, eleven cases out of eighteen showed a gradual fall of the value with the lapse of time, whereas four showed a fall at first and then a sudden rise afterwards. In three cases the value rose gradually at first with a subsequent fall.

In short, the tigrolytic values of C. S. F. rise after intra-cranial intervention, such as trauma and craniotomy, fall gradually in more than a month and finally return to normal. Some cases show up and down of the values during the course. This fluctuation does not accompany any clinical sign.

The period after intervention, at which the maximal value is reached, is uncertain. In two cases the maximal value was obtained two hours and six hours respectively after brain injury. In craniotomy

the maximal value was obtained after three hours and twelve hours respectively in two cases. Further details are not available at present.

#### (6) C. S. F. mixed with exudate and transudate.

When a part of a brain tumor is cystic, filled with yellow fluid, which is supposed to be transudate from blood vessels, the investigation of the fluid in twelve cases revealed the values to be 6-7 (+) in one case, 6-6 (+) in three, 6-5 (+) in two, 6-3 (+) in one; and 5-6 (+) in three cases, 5-5 (+) in one and 5-3 (+) in another one.

The fluid accumulating in the dead cavity in and outside the brain, which was formed after exstirpation of a tumor, is considered to be C. S. F. mixed with exudate. The fluid showed a high value in four cases; 6-4 (+), 5-7 (+), 5-6 (+) and 5-2 (+) respectively.

Also the subcutaneously accumulated bloody fluid following craniotomy C. S. F. mixed blood and exudate gave 6-7 (+) in one case, 6-3 (+) in one, 5-4 (+) in one and 5-0 (+) in two cases.

The C. S. F. containing no blood and accumulating subcutaneously by leakage (supposed to be mixed with exudate) showed 5-4 (+) in one case and 5-2 (+) in another. In the lymph accumulating subcutaneously, the values were 6-5 (+) and 5-4 (+).

The hematomatous fluid of chronic subdural hematoma, showed 6-7 (+). As to the plasma of the blood taken from elbow vein, one case was 6-7 (+) and three cases were 5-5 (+).

The examination of ascites in peritonitis tuberculosa (exudation fluid) and that in hepatic cirrhosis (transudation fluid) revealed 6-6 (+) and 6-3 (+) respectively.

Pus serum showed 5-7 (+), while bile and gastric juice 6-0 (+) and 5-0 (-) respectively, which were lower than normal.

#### 4. Summary and Discussions

(1) Mechanism of increase of enzyme action in C. S. F..

From the results of my study it is evident that the amount of tigrolytic enzyme in C. S. F. is small and that in blood plasma is considerably large.

The rise of the enzyme in C. S. F. seems to be brought about either by the transudation of the enzyme from the blood plasma into C. S. F. or by concentration of the enzyme in C. S. F..

(a) Transudation of the blood plasma into C. S. F.

The increase of tigrolytic action when the blood comes into C. S. F. is due mainly to the admixture of the blood plasma and partly to that of polymorphonuclear leucocytes. But when bleedings occurs at the time of lumbar puncture and the fluid becomes bloody, the nucleolytic value of C. S. F. is little changed. When the blood plasma is added to C.S.F. in vitro, the value rises very much, but when the whole blood is added to C. S. F. in such an amount as to color the fluid deeply red, it shows no much rise. (The amount the plasma contained in the added blood seems to be too small).

Experimentally 1.0cc. of blood was taken from auricular vein of a rabbit, and was mixed with 0.1cc. of 3.8% natrium citrate. Then the blood was injected into the spinal cavity of the same rabbit by cysternal puncture. Afterwards C. S. F. was taken every day by cysternal puncture, and examined on the tigrolytic value. It was 6-2 (+) on the first and the second day after injection. If 1.0cc. of the whole blood without natrium citrate is injected, it was 6-4 (+) on the eleventh day. In this case xanthochromia of C. S. F. continued for three weeks. The value of C. S. F. in normal rabbit was 6-0 (-) or 5-0 (-).

Thus bleeding into the cerebrospinal cavity is a cause of rise of the tigrolytic value of the fluid. But the rise seems to be due to a reactive inflammation of the meninges as well as to the extravasated blood itself. In this connection the following experiment was performed in rabbits. After cysternal puncture, aseptic meningitis was produced by injecting 1.0cc. of physiological saline containing 1/200g. sterile lycopodium. The cysternal C. S. F. was examined every day there after. Leucocyte count in C. S. F. reached the maximum (2700) on the second day and the tigrolytic value of the fluid continued to be 6-0 (+) from the second to the tenth day [control 6-0 (-)]. Xanthochromia of C. S. F. persisted until the fifteenth day. Thus it is evident that aseptic meningitis increases tigrolytic value of the

fluid to a certain extent.

The main reason may be the plasma outflow during inflammatory exudation. Ascites of men shows a high value whether it is exudate or transudate.

Supposing that the source of the enzyme in C. S. F. might also be disintegrated cerebral nerve cells, I examined the tigrolytic value of the homogenate of the human cerebral cortex. But it was quite normal. No change of the value was found even if the homogenate was added to C. S. F.. Therefore it is certain that the enzyme or its activator does not come from nerve cells.

(b) Concentration of C. S. F.

In matured dogs, obstructive internal hydrocephalus was produced by introducing a gelatine sponge into the fourth ventricle (Nishimura 1954), and the tigrolytic effect of C. S. F. in the lateral ventricle and that in the spinal cavity were compared. The value of the fluid in the lateral ventricle was 6-1 (+) and that in the spinal cavity 6-0 (+) in one case. In the other two cases it was 6-0 (+) in the former fluid and 6-0 (-) in the latter. Thus C. S. F. in the lateral ventricle gave a more or less higher value than that in the spinal cavity in case of obstructive hydrocephalus. This must be the result of concentration of stagnated C. S. F. in the lateral ventricle. The normal value was obtained in cases of hydrocephalus communicans congenitus in human beings. But when C. S. F. was temporarily stagnated inside the ventricle after intracranial operation, the values were usually higher.

If C. S. F. is removed by lumbar puncture and the secretion of C. S. F. is stimulated, the lowering of the values is observed. The reason may be the dilution of the fluid.

(2) Results of the test in cases of various diseases.

The normal value was 6-1 (+), 5-0 (-) and 5-0 (+). C. S. F. in most intracranial diseases showed values within this range.

In brain tumors, normal or lower values were found in all cases. There were no differences in the value according to the nature and localisation of the tumor.

Most cases of encephalitis japonica gave 6-2 (+) from the early stage of the disease. All cases of meningitis tuberculosa gave higher values, ranging from 6-3 (+) to 6-4 (+).

C. S. F. in meningism, abdominal typhus and ekiri showed values lower than 6-1 (+). Although the difference in value between these diseases and Japanese encephalitis is slight, the test may be helpful to a certain extent in the early diagnosis of encephalitis japonica.

Brain abscess gave a higher value, but only a slight rise of the value was found in intracerebral hemorrhage, probably as the result of the slighness of meningeal inflammation.

Many case of cerebral injury showed 6-3 (+) shortly after accident. The severer the injury, the longer the higher value persists. In cases of the fourth type of cerebral injury (intracranial hemorrhage type), fairly high values were obseved after

one year. Long continuing high values may have some relation to posttraumatic complaints. This is inferred from the fact that higher values are found in many cases of posttraumatic epilepsy and other posttraumatic disorders.

The C. S. F. after craniotomy shows a higher value than that in cerebral injury, sometimes reaching 6-6 (+), probably because bleeding and exudation take place more severely in case of craniotomy than in cerebral injury. Similarly it has been established that the more intense the xanthochromia of the fluid the higher the tigrolytic value and that relatively high values are frequently met with in bloody C. S. F.. It may be reasonable to assume that the smaller the magnitude of operation, the lower is the tigrolytic value of the fluid. Repeated lumbar punctures cause lowering of the value due to the dilution of C. S. F. by its increased secretion. The reason for the gradual lowering of the once heightened tigrolytic value with the lapse of time after accident or operation is in the absorption and exchange of C. S. F..

High values are observed in many cases of psychosis, a fact, which may explain the decrease of nucleic acid in cerebral nerve cells in cases of schizophrenia and manic depressive psychosis (Hydén 1947, Marui Suzuki 1948).

### (3) Examination with pure ribonuclease.

As ribonucleic acid is believed to be the principal constituent of Nissl body, a factor dissolving the body is naturally supposed to be ribonuclease.

Pyronin methyle green (Bracket 1940), which stains ribonucleic acid red, and desoxy-ribonucleic acid green, stains Nissl body red. Therefore the fact, that in the sections of the cats spinal cord which have been treated with C. S. F., Nissl body alone disappears, may be assumed to be the result of dissolution of ribonucleic acid. Both in Nissl's staining and in pyronin methyl green staining of the sections, the optimal pH value of C. S. F. is the same and the decrease in the tigrolytic effect of the fluid by multiple dilution is quite equally demonstrable.

When the sections are treated with pure ribonuclease solution, (manufactured by Minophagen Pharmaceutical Comp, Tokyo), instead of C. S. F., the disappearance of Nissl body occurs entirely in the same way as in the case of treatment with C. S. F.. The minimum amount of ribonuclease which causes tigrolysis in two 20 micra thick celloidine sections of the cat's spinal cord, corresponds, when calculated from multiple dilution of the ribonuclease solution to about 40 $\gamma$  (0.04 mg). of the pure ribonuclease powder.

Now, ribonucleic acid suspension is taken for the test instead of sections of the spinal cord (Minophagen Pharmaceutical Comp. Tokyo). 0.3cc. of distilled water, containing three mg. powder of ribonucleic acid, is added to each 0.5cc. of C. S. F. diluted in multiplication. The mixture is lightly shaken and kept at room temperature, then after about half a minute, white clouding of the ribonucleic acid suspension disappears and becomes transparent in the C. S. F. of lower dilution, but the clouding remains the same in the C. S. F. of higher dilution. The maximal dilution of C. S. F., which causes clearing of the suspension, agrees with that which causes tigrolysis in sections of the spinal cord. This is a simple and reliable method for

the demonstration of the presence of ribonuclease in C. S. F., but since nucleic acid is very expensive, it can not be routinely used.

From the above experiments it is evident that the enzymatic action of C. S. F. is caused by ribonuclease. The optimum pH 7.0-8.0 in the enzymatic action of C. S. F. coincides very well with the optimum pH of ribonuclease, vis. 7.7. But there is no positive evidence which excludes the possibility of the existence of other analogous enzymes.

### 5. Conclusion

1) Following E. A. Spiegel and others, the appearance of nuclease in C. S. F. after cerebral concussion was reexamined.

2) A quantitative method for practical use was devised by improving their method and tigrolytic effects of C. S. F. in various diseases of the central nervous system were studied.

3) Nuclease action can be found not only in C. S. F. after cerebral concussion, but also in normal C. S. F., though slightly.

4) C. S. F. in brain tumors shows usually a lower value than normal.

5) The value is high in cases of intracranial inflammation and bleeding.

6) Most cases of encephalitis japonica give a slight but definite rise of the value and this may be helpful for early diagnosis of the disease.

7) After craniotomy, the value rises shortly after operation and returns to normal in the course of time. The less the magnitude of operation, the less the rise of the tigrolytic value of the fluid.

8) After cerebral injuries, the value rises immediately and returns to normal gradually. The severer the injuries, the longer the rise persists.

9) Idiopathic epilepsy gives the normal value, but posttraumatic epilepsy and other posttraumatic disorders show fairly high values.

10) The values in many cases of psychosis and neurosis are fairly high.

11) In cases of intracranial bleeding and inflammation and of stagnation of C. S. F., there occurs an increase in nuclease action of C.S.F.. The nuclease is supposed to originate largely from the blood plasma.

12) The enzyme concerned is mainly ribonuclease.

13) Nissl body can be stained in celloidine sections at any pH of C. S. F., while the body can not be stained in other embedding media at pH above 6.0 of C. S. F..

At the conclusion, I should express my hearty thanks to Prof. Chisato Araki. Dr. Shunji Tokuoka, and Dr. Akira Inamoto for their kind guidance. I am also deeply indebted to Municipal Kyoto Hospital, Shijoomiya Hospital, Psychiatry and Surgery Departments of Kyoto University Hospital for their kind cooperation.



## Explanation of Plates

- Fig. 1** Anterior horn cells of a normal cat. Nissl bodies are normally stained. × 600
- Fig. 2** The same as the above. × 150
- Fig. 3** Complete tigrolysis caused by enzymatic action of cerebrospinal fluid. Nucleolus and adjacent glia nuclei are rather well preserved. × 600
- Fig. 4** The same as the above. × 150
- Fig. 5** Incomplete tigrolysis caused by enzymatic action of C.S.F.. Nissl bodies are still preserved at the periphery of the cytoplasm. × 600
- Fig. 6** In a series of test tubes containing C. S. F. of multiple dilution (1 : 2), a suspension of ribonucleic acid is added and the disappearance of the clouding of the suspension is observed. Dilution degree of C. S. F. is 1/1, 1/2, 1/4, 1/8, 1/16 from left to right.

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## 和文抄録

## 髄液の核酸融解酵素作用の臨床的諸様相

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大学院学生 千 原 卓 也

1) 脳振盪後の髄液中にヌクレアーゼが出現すると言う E. A. Spiegel 等の実験 (1946) を追試した。

2) 彼の検査方法を検討改変して、簡便なる定量法を考案し、臨床的応用を計るべく、一般臨床疾患につき検査してみた。その方法は、髄液の pH を一定にしその倍数希釈を行い、正常猫脊髓のツエロイゲン切片に作用せしめ、同切片前角細胞ニツスル小体のチグロリージス像判定により、髄液の酵素作用強度を決定した。

3) ヌクレアーゼ作用は脳振盪受傷後の髄液中のみならず、健常髄液中にもある。

4) 脳腫瘍の髄液では健常値より低いものが多い。

5) 脳に炎症、出血ある場合は高くなる。

6) 日本脳炎では pH 6.0 で  $2^2$  (+) の値をとるもの多く、早期鑑別診断の一助となる可能性がある。

7) 開頭術後、値は上昇し日数経過と共に健常値に

回復する。手術侵襲の軽度なる程、値の上昇も少い。

8) 脳外傷後、値は上昇し日数経過と共に健常値に回復する。外傷程度の大なるもの程健常値に回復するまでの期間は長い。

9) 脳腫瘍は健常値である。しかし外傷性脳腫瘍、外傷後胎症ではやゝ高い値を示す。

10) 精神病、神経症には値の稍高いものが多い。

11) 髄液中にヌクレアーゼが出現するのは頭蓋内に出血、炎症、髄液循環障害のある場合であつて、血漿中より移行するものと思われる。脳神経細胞より出現する事は認めなかつた。

12) この酵素は主としてリボヌクレアーゼである。

13) ニツスル小体は pH 6.0 以上では染色像に現われて来ないが、ツエロイゲン切片では pH と無関係に之を染め出し得る。

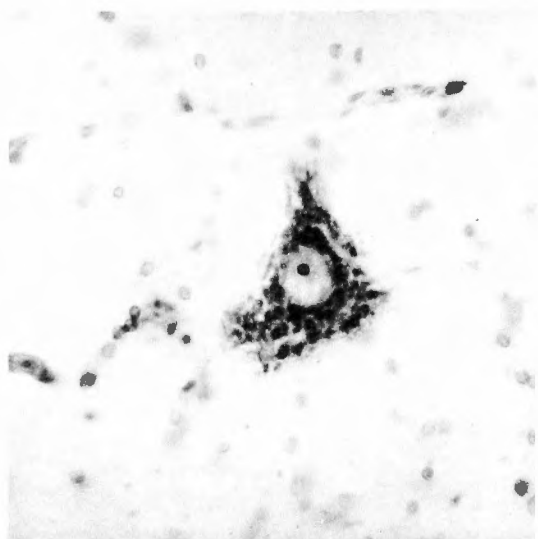


Fig 1

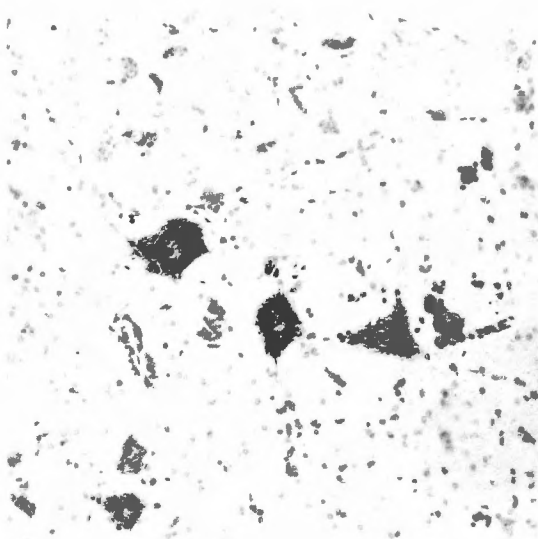


Fig 2

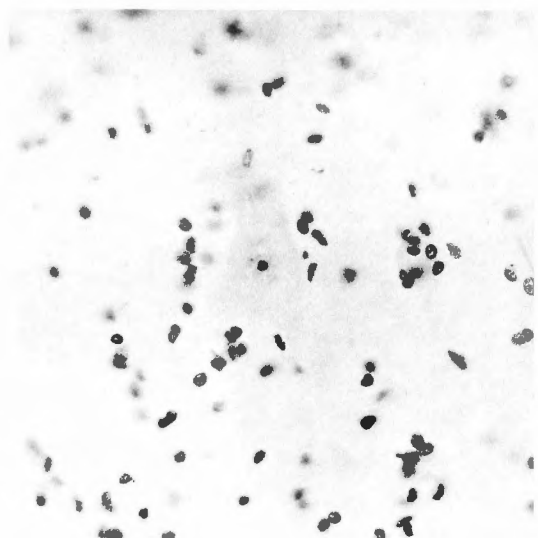


Fig 3

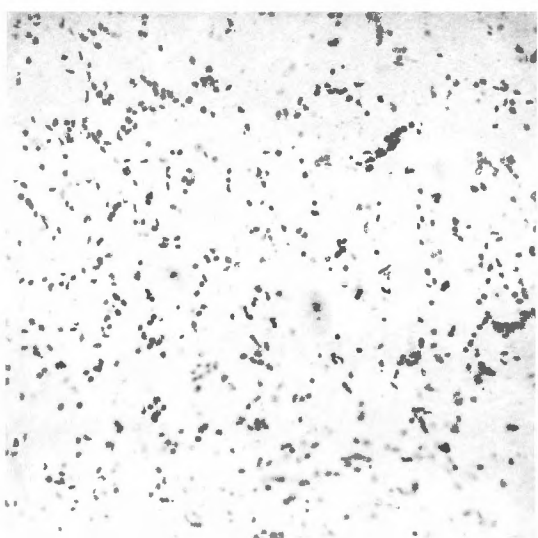


Fig 4

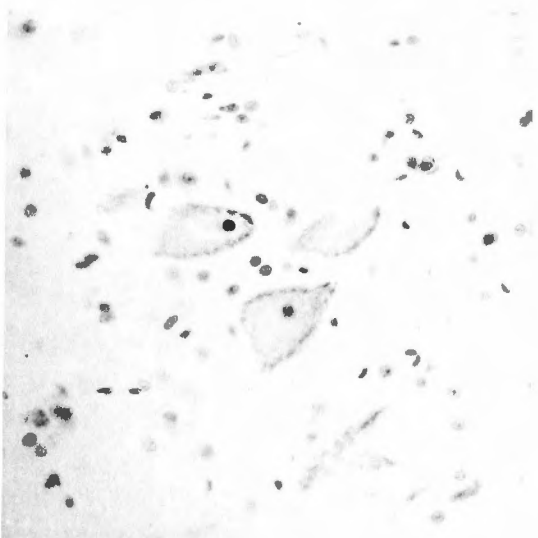


Fig 5

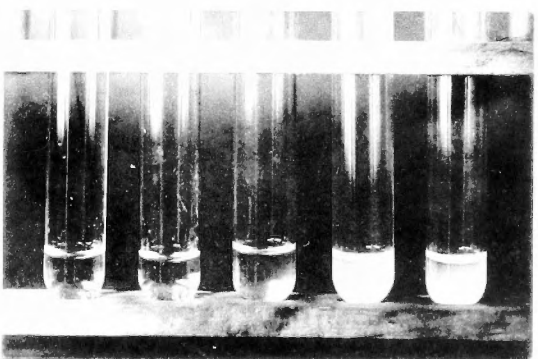


Fig 6